[CANCER RESEARCH 52, 4965–4968, September 15, 1992]

Effects of Hyperthermia on Blood Flow and *cis*-Diamminedichloroplatinum(II) Pharmacokinetics in Murine Mammary Adenocarcinomas

P. L. Ausmus, A. V. Wilke, and D. L. Frazier[1]

Department of Environmental Practice, University of Tennessee, College of Veterinary Medicine, Knoxville, Tennessee

ABSTRACT

The effect of localized hyperthermia on blood flow and *cis*-diamminedichloroplatinum(II) (CDDP) pharmacokinetics in 7,12-dimethylbenz[*a*]anthracene-induced mammary adenocarcinomas was studied. Blood flow was determined in rat tumors and normal tissue immediately and 1, 2, and 3 h after local hyperthermia treatment (43°C, 1 h) as well as in unheated tumors of rats. The rate of blood flow in the tumor was increased 1.9 times at the end of treatment relative to control values and returned to the control values by 3 h after hyperthermia. Similarly, the rate of blood flow in the peripheral skin around the tumor immediately after hyperthermia was 2.2 times greater than that of unheated skin and returned to near normal values by 3 h after heating.Tumor-bearing rats received CDDP 1 h before, at the beginning of, at the end of, and 1 h after hyperthermia administration. The CDDP plasma concentration *versus* time profiles for rats did not vary statistically between treatment groups. Two h after CDDP administration, the mean tumor CDDP concentration of the rats which received drug at the beginning of hyperthermia was statistically greater ($P < 0.05$) than tumor CDDP concentrations in rats which received drug at the end of heat treatment. The latter group was given CDDP when tumor blood flow was the greatest; however, mean tumor drug concentration was lowest of all the groups. The mean drug concentration in tumor tissues of rats which received drug 1 h after hyperthermia was comparable to rats which received drug at the beginning of hyperthermia. This suggests that drug delivery or uptake in tumors may be altered when local hyperthermia is administered concurrently or sequentially.

INTRODUCTION

CDDP[2] is used predominantly in the treatment of ovarian and testicular cancer. In addition, CDDP is currently being used for the treatment of head and neck cancers, osteosarcomas, leukemia, and tumors of the lung, skin, and breast (1). This anticancer drug binds to DNA, inactivating it as a template for replication and transcription (2). It has been demonstrated using clonogenic assays (3) and thermochemosensitivity screening (4) that heat plus a variety of chemotherapeutic agents, including CDDP, are synergistic for cell killing.

Several studies suggest that synergism may depend on sequencing of CDDP relative to hyperthermia. *In vivo* studies (5, 6) suggest that heat and drug must be administered close together in time in order to obtain synergistic killing. Similarly, thermal enhancement of cytotoxicity by CDDP in the thermoresistant melanoma line M14 was demonstrated only when the two modalities were given close together or simultaneously (7). Synergism was achieved when hyperthermia preceded CDDP; however, this regimen was less effective than simultaneous administration at impairing colony-forming ability. In contrast, experiments combining radiation with heat and CDDP (8) showed the greatest delay in regrowth of implanted FSallC fibrosarcoma cells to occur when CDDP preceded hyperthermia and radiation.

Synergism between hyperthermia and CDDP may result from increased intracellular drug concentrations. Localized hyperthermia induces an increase in blood flow accompanied by dilation of vessels and an increase in permeability of the vascular walls in tissue (9, 10). These alterations could increase CDDP delivery to tumor tissue or increase intracellular concentrations of CDDP via altered membrane permeability. In the present study, the changes in blood flow and CDDP pharmacokinetics in mammary adenocarcinomas of Sprague-Dawley rats were compared at various times relative to local hyperthermia.

MATERIALS AND METHODS

Experimental Design. Each treatment group consisted of 7–8 female Sprague-Dawley rats with mammary adenocarcinomas (1 cm in diameter) induced by p.o. administration of 7,12-dimethylbenz[*a*]anthracene. The primary, chemically induced mammary adenocarcinomas were confirmed histopathologically. Rats received HT only, CDDP only, HT plus CDDP, or neither. Blood flow was measured in rats treated with HT at the end of HT or 1, 2, or 3 h after HT. Rats given HT plus CDDP received CDDP 1 h before, at the beginning of, at the end of, or 1 h after HT. Rats receiving CDDP were euthanized 2 h after drug administration for measurement of tumor drug concentrations or blood flow.

Hyperthermia Treatment. Local hyperthermia (43°C) was induced using a Surgical Laser Technology Nd:YAG laser system (10 W; 1064 nm). Rats were anesthetized with ketamine (6.25 mg) and acepromazine (0.125 mg) i.m. The energy was focused on the tumor via a fiber with the tip placed 10–12 cm above the tumor surface in order to illuminate only the circumference of the tumor. The laser was interfaced to a computer and thermometry unit which provided feedback to control the tumor temperature. Temperature was monitored by thermocouples placed superficial to, in the middle of, and deep to each tumor. Superficial cooling of the skin was achieved using forced moist oxygen flow from a nebulizer when necessary.

CDDP Analysis. CDDP (Platinol) was obtained from Bristol Laboratories and was prepared by adding sterile H_2O to achieve a final concentration of 1 mg/ml. At various times relative to hyperthermia, CDDP (10 mg/kg) was administered via a catheterized tail vein. Blood (0.7 ml) was collected via the lateral tail vein immediately, 30 min, and 1 and 2 h after CDDP administration. The small sample size allowed analysis of only the plasma ultrafitrate. Samples were centrifuged at 2500 rpm (10 min at 4°C), plasma was transferred to an ultrafiltration membrane (Amicon 2100, CF-50) and centrifuged at 3500 rpm (30 min at 4°C). The resulting ultrafiltrate containing only the platinum fraction not bound to plasma proteins was stored at −70°C until analysis.

Flameless atomic absorption spectroscopy was used to determine the amount of platinum in the ultrafiltrate of rat plasma according to the method described by El-Yazigi and Al-Saleh (11). Samples were diluted 1:10 with 0.2% nitric acid. The furnace temperature program consisted of drying at 120°C, charring at 750°C and 900°C, and a 4-s atomization stage (2000°C). The absorption of the atomized platinum was measured at 265.9 nm.

Tissue samples were kept frozen until analysis. Tissues were prepared according the method described by McGahan and Tyczkowska (12). Tumor samples (200 mg) were incubated overnight at room temperature in 0.5 ml of concentrated nitric acid. After 5 min of boiling, the

Received 2/13/92; accepted 7/8/92.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom requests for reprints should be addressed, at University of Tennessee, College of Veterinary Medicine, P. O. Box 1071, Knoxville, TN 37996.

[2] The abbreviations used are: CDDP, *cis*-diamminedichloroplatinum; HT, hyperthermia; AUC, area under the curve.

resulting clear yellow solution was analyzed directly by atomic absorption spectroscopy without dilution.

Pharmacokinetic parameters were determined using an automated curve-stripping program (R-Strip). Calculation of parameters was as follows:

$A = Cp_0$ = extrapolated drug concentration in the central compartment at time = 0

B = y intercept of the elimination phase

α = Slope of the distribution phase

β = Slope of the elimination phase

$t_{1/2}\alpha = \ln 2/\alpha$

$t_{1/2}\beta = \ln 2/\beta$

CL = Dose/AUC

Vd_c = Dose/Cp_0

$$Vd_{ss} = \text{Dose}\,\frac{[(A/\alpha^2) = (B/\beta^2)]}{[(A/\alpha) + (B/\beta)]^2}$$

AUC was calculated by the trapezoidal method (13). Vd_c and Vd_{ss} represent volumes of distribution of the central compartment and steady state, respectively. CL is the whole body clearance; $t_{1/2}\alpha$ and $t_{1/2}\beta$ are the half-lives of the distribution and elimination phases, respectively. Statistical comparison of these parameters was performed using the General Linear Model (SAS, Cary, NC). Relationships between tumor CDDP concentrations and plasma CDDP pharmacokinetics were evaluated using Pearson correlation coefficients.

Determination of Blood Flow. Blood flow in the tumor and overlying skin was measured using a modification of the reference method described by Malik *et al.* (14). The left ventricle was catheterized through the right common carotid artery. Another catheter was inserted into an internal iliac artery. Approximately 12,000 radiolabeled microspheres (^{113}Sn), suspended in 0.46 ml saline, were injected through the ventricular catheter over a period of 10 s and then flushed with 0.15 ml saline. Reference blood samples (0.03 ml/min beginning at the start of injection and continuing for 70 s) were withdrawn from the iliac arterial catheter using a withdrawal pump (Sage Instruments). Blood flow was calculated from the measured activity (cpm) according to the relation

$$\text{Blood flow} = \frac{(\text{Tissue cpm})(\text{ref blood wt})}{(\text{Ref blood cpm})(\text{tissue wt})}$$

RESULTS

The Nd:YAG laser technique achieved temperature profiles believed to be in the therapeutic range (15) in approximately 87% of the rat tumors. Blood flow and CDDP concentrations were evaluated only in rats in which temperatures achieved at least 42°C in the center of the tumor. Temperatures just beneath the skin and superficial to the tumor were the most variable. Average temperatures superficial to, in the center of, and deep to the tumor of all rats used in the study were 42.0 ± 2.8 (SEM), 43.4 ± 0.5, and 42.4 ± 1.3, respectively.

There was a marked increase in tumor blood flow by the end of hyperthermia treatment; however, this increase was not statistically significant (Fig. 1). Flow returned to approximately control values by 3 h posttreatment. Blood flow was statistically greater ($P < 0.05$) in the skin above tumors at the end of hypertheria than in the skin above tumors which were not heated. Blood flow did not differ between rats treated with hyperthermia alone compared to those treated with hyperthermia plus CDDP.

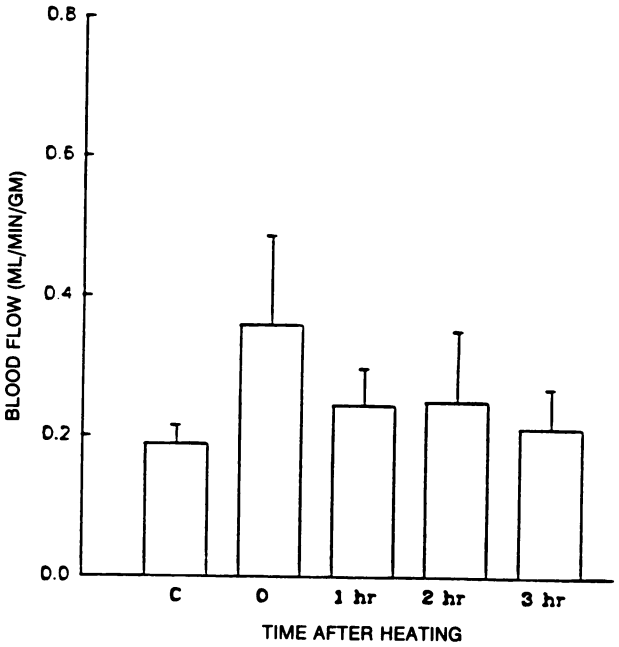

Fig. 1. Blood flow of tumors (ml/min/g) in control rats (*C*) and rats given local hyperthermia treatment (43°C, 1 h) at the end of (0) and 1, 2, or 3 h after heating. *Bars*, SEM.

The predicted CDDP concentration *versus* time profiles in plasma ultrafiltrate based on the intercepts (A and B) and rate constants (α and β) for each treatment group are shown in Fig. 2. The distribution half-life ($t_{1/2}\alpha$) of the group receiving CDDP only was significantly shorter than the group receiving CDDP 1 h before, at the beginning of, and at the end of hyperthermia. The elimination half-life ($t_{1/2}\beta$) of the group receiving CDDP 1 h before hyperthermia was significantly greater than those of all other groups. The Vd_{ss} was significantly greater ($P < 0.05$) in the group receiving drug 1 h before hyperthermia than in the groups that were dosed at the beginning of, end of,

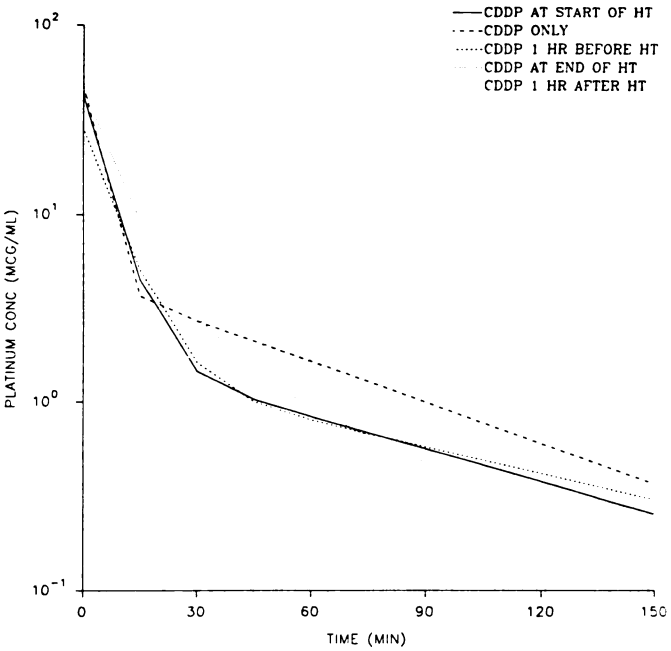

Fig. 2. Average free CDDP concentration *versus* time profiles for rats given CDDP only or CDDP 1 h before HT, at the beginning of HT, at the end of HT, or 1 h after the end of HT.

or 1 h after hyperthermia. Clearance was greatest in the group receiving CDDP at the end of heat; however, this difference was statistically significant only when compared to the group in which CDDP was administered 1 h after hyperthermia (Table 1).

Several plasma CDDP pharmacokinetic parameters were correlated to tumor drug concentration. Mean tumor drug concentration was significantly greater ($P < 0.05$) in rats receiving drug at the beginning of heat compared to those receiving drug at the end of heat (Fig. 3). Tumor drug concentrations were found to be directly correlated to the AUC ($r = 0.721$, $P < 0.0001$) and peak plasma concentration (A intercept)($r = 0.353$, $P < 0.0295$). Although the AUC was not statistically different between treatment groups, the group receiving the drug at the end of HT had the lowest AUC value (Table 1). Tumor drug concentration was indirectly correlated to clearance ($r = -0.528$, $P < 0.0006$) and the volume of distribution of the central compartment (Vd_c) ($r = -0.427$, $P < 0.0075$).

Within the range of tumor sizes treated in the present study, there was no correlation between tumor drug concentration and tumor size. All rats were treated when their tumors were approximately 1 cm in diameter (0.67 ± 0.03 cm^3) and tumor sizes did not differ overall between treatment groups.

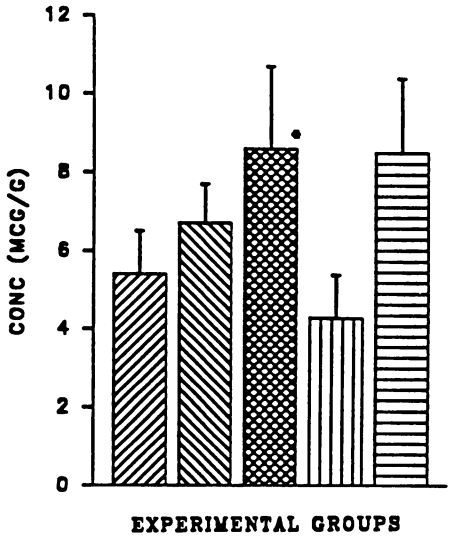

Fig. 3. Platinum concentrations (μg/g) of tumors in murine mammary adenocarcinomas treated with cisplatin and/or hyperthermia. *Bars*, SEM. Measurements were taken 2 h after drug administration. *, statistically ($P = 0.05$) greater than the group in which drug was administered at the end of hyperthermia. ▨, drug only; ▧, drug 1 h before HT; □, drug at start of HT; ▥, drug at end of HT; □, drug 1 h after HT.

DISCUSSION

The results of the present study are in general agreement with previous reports that demonstrate that hyperthermia induced increases in blood flow in skin and tumor tissue with the increase in skin being greater than in the tumor (16). In contrast to other studies (17, 18), marked decline in tumor blood flow was not seen following hyperthermia. It is possible that 7,12-dimethylbenz[*a*]anthracene-induced tumors are more thermotolerant than implanted tumors. The relatively mild thermal dose used in this study may also explain why a marked decline in tumor blood flow was not seen. Variations in blood flow measurement techniques and tumor location and size at time of treatment are additional differences between studies.

Rats having the greatest peak plasma ultrafiltrate CDDP concentrations also had the greatest tumor CDDP concentrations. An increase in Vd_c correlated to a decrease in tumor CDDP concentrations consistent with retention of drug in blood and the highly perfused organs compared to distribution of drug to lightly perfused tumor tissue. As expected, an increase in the overall elimination of drug (CL) in rats was consistent with decreased delivery of CDDP to tumors. To our knowledge, other studies have not yet evaluated plasma pharmacokinetic changes associated with local hyperthermia; however, changes have been shown to occur with whole body hyperthermia. Riviere *et al.* (19) reported a significant increase in the rate constant reflecting the decay of free CDDP and CL at 43°C compared to 37°C. They also demonstrated that canines subjected to whole-body heating (42°C for 1 h) after receiving CDDP (1 mg/kg) had an increased rate of transformation of reactive metabolites from parent CDDP and increased volume of distribution of free platinum. Increased CL was attributed to increased renal elimination, increased biotransformation, or increased rate of tissue binding.

Wilke *et al.* (20) reported a decrease in peak plasma concentrations of Adriamycin (30 mg/m^2) in normal dogs when a whole-body hyperthermia treatment (42°C for 1 h) was administered along with the drug. They also demonstrated a significant increase in the apparent volumes of distribution (Vd) and a significant decrease in AUC when Adriamycin and whole-body hyperthermia where administered concurrently.

The present study suggests that the greatest tumor drug concentration may be achieved when drug is administered at the beginning of or 1 h after hyperthermia treatment. The lowest tumor drug concentrations were seen in rats that were given drug at the end of a 1-h hyperthermia treatment. This group

Table 1 *Plasma ultrafilterable CDDP pharmacokinetic parameters in rats administered CDDP with or without heat (mean ± SEM)*

	Time of CDDP administration relative to hyperthermia				
	Drug only	1 h before HT	Beginning of HT	End of heat	1 h after HT
A (μg/ml)	43 ± 9.8	27 ± 3.3	43 ± 15	41 ± 23	51 ± 18
$t_{½}\alpha$ (h)	0.06 ± 0.02[a]	0.17 ± 0.01[b]	0.12 ± 0.02	0.13 ± 0.02	0.11 ± 0.02
$t_{½}\beta$ (h)	0.95 ± 0.28	3.2 ± 1.1[c]	0.93 ± 0.35	1.20 ± 0.33	1.10 ± 0.37
AUC (μg/ml/h)	436 ± 64	460 ± 70	568 ± 169	332 ± 48	546 ± 88
CL (ml/kg/min)	5.7 ± 0.07	6.1 ± 0.72	6.7 ± 1.2	8.3 ± 1.2[d]	4.9 ± 0.78
Vd_{ss} (liters/kg)	0.34 ± 0.11	0.81 ± 0.35[e]	0.16 ± 0.04	0.13 ± 0.02[f]	0.07 ± 0.01
Vd_c (liters/kg)	0.08 ± 0.02	0.10 ± 0.01	0.10 ± 0.02	0.28 ± 0.06	0.18 ± 0.06
Tumor concentration (μg/g)	5.4 ± 1.1	6.7 ± 1.0	8.6 ± 2.1[g]	4.3 ± 1.1	8.5 ± 1.9

[a] Significantly shorter ($P < 0.05$) than groups given CDDP 1 h before, at the beginning of, or end of HT.
[b] Significantly longer ($P < 0.05$) than groups given CDDP only, or CDDP at the beginning or 1 h after HT.
[c] Significantly longer ($P < 0.05$) than all other groups.
[d] Significantly greater ($P < 0.05$) than group given CDDP 1 h after HT.
[e] Significantly greater ($P < 0.05$) than groups given CDDP at the beginning of, end of, or 1 h after HT.
[f] Significantly greater ($P < 0.05$) than group given CDDP 1 h after HT.
[g] Significantly greater ($P < 0.05$) than group given CDDP at the end of HT.

also had the lowest AUC and significantly greater Vd_c and CL. Blood flow in the tumor was shown to be increased at the end of hyperthermia. This suggests but does not prove that decreased tumor drug concentrations result when blood flow is increased, presumably due to a wash-out effect where increased flow allows inadequate time for drug uptake into the cells. Tumor drug concentrations were greatest in rats given CDDP at the beginning of hyperthermia. Altered membrane permeability may precede blood flow changes and favor drug uptake.

A limitation of the present study is that CDDP concentrations were measured in tumors at only one point in time. It is possible that drug concentrations would differ between treatment groups at times other than 2 h after drug administration. CDDP binds to DNA and can persists in tumor tissue for many weeks (21) suggesting that differences in drug concentrations would persist between treatment groups. Previous *in vitro* studies suggested that synergistic killing requires that heat and drug be administered close together in time, if not simultaneously (4, 5). This is consistent with increased drug concentrations seen in the present study. No attempt was made to evaluate cytotoxicity of the sequencing protocols described in the present study. Resistance to CDDP cytotoxicity has been noted in some tumor cell lines in association with significant CDDP concentrations (22). Certain cells may tolerate the presence of CDDP and not be affected by increased intracellular CDDP concentrations. Concurrent exposure to CDDP and HT may alter this tolerance by interfering with DNA repair. Studies are needed which evaluate the relationship between synergistic killing and CDDP concentrations in sensitive and resistant tumors.

REFERENCES

1. Litterst, C. L., Gram, T. E., Dedrick, R. L., Leroy, A. F., and Guarino, A. M. Distribution and disposition of platinum following intravenous administration of *cis*-diamminedichloroplatinum(II) (NSC 119875) to dogs. Cancer Res., *36:* 2340–2344, 1976.
2. Roberts, J. Cisplatin. *In*: B. A. Chabner and H. M. Pinedo (eds.), The Cancer Pharmacology Annual, pp. 95–117: Amsterdam; Excerpta Medica, 1983.
3. Hahn, G. M., Braun, J., and Har-Kedar, I. Thermochemotherapy: synergism between hyperthermia (42–43°C) and Adriamycin (or bleomycin) in mammalian cell inactivation. Proc. Natl. Acad. Sci. USA, *72:* 937–940, 1975.
4. Calabro, A., Singletary, S. E., Tucker, S., Boddie, A., Spitzer, G., and Cavaliere, R. *In vitro* thermochemosensitivity screening of spontaneous human tumors: significant potentiation for cisplatin but not Adriamycin. Int. J. Cancer, *43:* 385–390, 1989.
5. Herman, T. S., Jochelson, M. S., Teicher, B. A., Scott, P. J., Hansen, J., Clark, J. R., Pfeffer, M. R., Gelwan, L. E., Molnar-Griffin, B. J., Fraser, S. M., Svennson, G., Bornstein, B. A., Ryan, L., and Coleman, C. N. A phase I-II trial of cisplatin, hyperthermia and radiation in patients with locally advanced malignancies. Int. J. Radiat. Oncol. Biol. Phys., *17:* 1273–1279, 1989.
6. Marmor, J. B. Interactions of hyperthermia and chemotherapy in animals. Cancer Res., *39:* 2269–2276, 1979.
7. Greco, C., Cavaliere, R., Giovanella, B., Natali, P. G., and Zupi, G. Effect of sequential application of hyperthermia and chemotherapy on the survival of a thermoresistant human melanoma cell line. Cancer Biochem. Biophys., *9:* 223–232, 1987.
8. Herman, T. S., and Teicher, B. A. Sequencing of trimodality therapy [*cis*-diamminedichloroplatinum(II)/hyperthermia/radiation] as determined by tumor growth delay and tumor cell survival in the FSaIIC fibrosarcoma. Cancer Res., *48:* 2693–2697, 1988.
9. Dewey, W. C., Highfield, D. P., Freeman, M. L., Cross, R. A., Wong, R. S. L., and Barrau, M. D. Cell biology of hyperthermia and radiation. *In*: S. Okada, M. Imamura, T. Terashima, and H. Yamaguchi (eds.), Proceedings of the Sixth International Congress of Radiation Research, pp. 832–839. Tokyo: H. Tappan Printing Co., 1979.
10. Dickson, J. A., and Calderwood, S. K. Temperature range and selective sensitivity of tumors to hyperthermia: a critical review. Ann. NY Acad. Sci., *335:* 180–205, 1980.
11. El-Yazigi, A., and Al-Saleh, I. Rapid determination of platinum by flameless atomic absorption spectrophotometry following the administration of cisplatin to cancer patients. Drug Monitor, *8:* 318–320, 1986.
12. McGahan, M. C., and Tyczkowska, K. The determination of platinum in biological materials by electrothermal atomic absorption spectroscopy. Spectrochim. Acta, *42B:* 665–668, 1987.
13. Gibaldi, M., and Perrier, D. Estimation of areas. *In*: J. Swarbrick (ed.), Drugs and the Pharmaceutical Sciences, Vol. 15, pp. 445–449. New York: Marcel Dekker, Inc., 1982.
14. Malik, A. B., Kaplan, J. E., and Seba, T. M. Reference sample method for cardiac output and regional blood flow determination in the rat. J. Appl. Physiol., *40:* 472–475, 1976.
15. Song, C. W., Kang, M. S., Rhee, J. G, and Levitt, S. H. Effect of hyperthermia on vascular function in normal and neoplastic tissues. Ann. NY Acad. Sci., *335:* 35–46, 1980.
16. Song, C. W. Effect of local hyperthermia on blood flow and microenvironment: a review. Cancer Res., *44:* 4721s–4730s, 1984.
17. Rappaport, D. S., and Song, C. W. Blood flow and intravascular volume of mammary adenocarcinoma 13762A and normal tissues of rat during and following hyperthermia. Int. J. Radiat. Oncol. Biol. Phys., *9:* 539–547, 1983.
18. Song, C. W. Effect of hyperthermia on vascular functions of normal tissues and experimental tumors: brief communication. J. Natl. Cancer Inst., *60:* 711–713, 1978.
19. Riviere, J. E., Page, R. L., Dewhirst, M. W., Tyczkowska, K., Thrall, D. E. Effect of hyperthermia on cisplatin pharmacokinetics in normal dog. Int. J. Hyperthermia, *2:* 351–358, 1986.
20. Wilke, A. V., Jenkins, C. Milligan, A. J. Legendre, A., and Frazier, D. L. Effect of hyperthermia on normal tissue toxicity and on Adriamycin pharmacokinetics in dogs. Cancer Res., *51:* 1680–1683, 1991.
21. Poirier, M. C., Reed, E., Zwelling, L. A., Ozols, R. F., Litterst, C. L., and Yuspa, S. H. Polyclonal antibodies to quantitate *cis*-diamminedichloroplatinum(II)-DNA adducts in cancer patients and animal models. Environ. Health Perspect., *62:* 89–94, 1985.
22. Richon, V. M., Schulte, N., and Eastman, A. Multiple mechanisms of resistance to *cis*-diamminedichloroplatinum(II) in murine leukemia L1210 cells. Cancer Res., *47:* 2056–2061, 1987.